QTLs Affecting α-Tocotrienol, α-Tocopherol, and Total Tocopherol Concentrations Detected in the Ogle/TAM O-301 Oat Mapping Population

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ABSTRACT

Consumption of oat (*Avena sativa* L.) products has been credited with reducing the risk of various diseases. This may be due in part to tocopherol content. Studies have shown variation in α-tocotrienol, α-tocopherol, and total tocopherol levels among oat cultivars, however, the genetic basis of these traits is unknown. The objectives of this study were to examine the genetic mechanisms affecting tocopherol levels in the Ogle1040/TAM O-301 population and to identify quantitative trait loci (QTLs) useful for improving tocopherol levels in oat. The population was grown in Aberdeen and Tetonia, ID, over 4 yr. Alpha-tocotrienol, α-tocopherol, and total tocopherol contents were measured in harvested seed. Mean levels of all three tocopherol measurements were higher from the irrigated Aberdeen location than from the nonirrigated Tetonia location. One major and four minor QTLs were detected, which accounted for a majority of the α-tocotrienol variation across all locations, while six minor QTLs accounted for a majority of the α-tocopherol variation. Only one QTL affecting total tocopherol was detected apart from QTLs affecting α-tocotrienol and α-tocopherol. Overall, we have identified markers linked to QTLs affecting α-tocotrienol and α-tocopherol. Since the highest level of both tocopherols in lines of the population were higher than from previously tested cultivars, these QTLs should be useful to increase specific tocopherol levels.

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Abbreviations: AFLP, amplified fragment length polymorphism; CIM, composite interval mapping; HPLC, high-performance liquid chromatography; LG, linkage group; LOD, likelihood of odds; MAS, marker-assisted selection; OT, Ogle1040/TAM O-301; PCR, polymerase chain reaction; QTL, quantitative trait locus; RFLP, restriction fragment length polymorphism; RIL, recombinant inbred line; SNP, single nucleotide polymorphism; STS, sequence tagged site.

Oat (*Avena sativa* L.) is recognized as a healthful food, and a reduced risk of cardiovascular disease and lower cholesterol have been attributed to diets containing oat products (Welch, 1995). Much of the cholesterol-lowering capacity is attributed to the high soluble fiber (β-glucan) content in oat. There are, however, other phytonutrients that likely contribute to the overall health benefits of oat consumption. The high levels of α-tocotrienol and α-tocopherol found in oat are of note because of their antioxidant activity and links to prevention of cardiovascular diseases (Peter et al., 1997), Alzheimer disease (Zandi et al., 2004), glaucoma (Engin et al., 2007), and prostate cancer (Helzlsouer et al., 2000).

The tocochromanols or tocopherols are a group of lipophilic antioxidants found in plants. These metabolites are grouped into two main classes, tocotrienols and tocopherols, based on their prenyl side chains. Tocotrienols have an unsaturated geranylgeranyl side chain whereas the tocopherols have a saturated phytyl side chain. Each class consists of four principal homologs termed alpha,

Published in Crop Sci. 48:2141–2152 (2008).
doi: 10.2135/cropsci2007.11.0658

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beta, gamma, and delta, depending on the methylation pattern on the chromanol ring. Alpha-tocopherol has the highest vitamin E activity of all the tocopherols, as accessed by the resorption-gestation test in rats (Leth and Søndergaard, 1977). The primary reason for this is preferential transportation of α-tocopherol to the peripheral tissues of mammals (Bramley et al., 2000). Although the vitamin E activity of tocotrienols is substantially lower than that of α-tocopherol, they have been shown to reduce cholesterol levels in chickens (Qureshi et al., 1986) as well as in humans and other mammals (Pearce et al., 1992; Theriault et al., 1999). The mechanism of action appears to be mediated through the prenyl side chain and results from the proteolytic degradation of 3-hydroxy-3-methyl-glutaryl-CoA reductase (Theriault et al., 1999). In addition, Theriault et al. (2002) showed that α-tocotrienol reduced endothelial leukocyte adhesion molecule expression and monocytic cell adherence, both of which have been associated with atherosclerosis and risks of myocardial infarction in humans (Peter et al., 1997; Ridker et al., 1998).

Nuts are some of the richest sources of tocopherols in the human diet, including almonds [*Prunus dulcis* (P. Mill.) D.A. Webber], hazelnuts (*Corylus avellana* L.), and peanuts (*Arachis hypogaea* L.). Among the cereals, wheat (*Triticum aestivum* L.) is particularly rich in tocopherols (Bramley et al., 2000), while barley (*Hordeum vulgare* L.) and oat have moderate amounts. In oat, the two most abundant tocopherols are α-tocotrienol and α-tocopherol with α-tocotrienol being the most abundant of the two (Barnes, 1983).

The few studies done in oat have shown that tocopherol content varies. Peterson and Qureshi (1993) found that in 12 oat cultivars total tocopherol concentrations in the grain ranged from 19 to 31 mg kg^{-1}. In a more recent study using 33 oat cultivars over nine location-years, Peterson et al. (2005) found mean concentrations of α-tocotrienol and α-tocopherol ranging from 13.3 to 24.4 mg kg^{-1} and 6.6 to 9.1 mg kg^{-1}, respectively. Although these reports indicate significant genotypic variation of tocopherol in oat, no studies have been done to determine the genetics of tocopherol inheritance. The objective of this study was to use an existing Ogle1040/TAM O-301 (OT) linkage map and mapping population (Portyanko et al., 2001) to evaluate genetic components affecting α-tocotrienol, α-tocopherol, and total tocopherols in whole grain oat.

MATERIALS AND METHODS

Plant Materials and Field Experiments

The $F_{6:8}$ OT recombinant inbred line (RIL) population previously used to construct one of the major oat linkage maps (Portyanko et al., 2001) was used in this study. The population consists of 136 RILs and was developed via single seed descent to the F_6 generation from which single panicle selections were made to develop F_7 lines. Bulked seed from each line was subsequently increased to produce enough $F_{6:8}$ seed for multiple evaluations.

Parents and RILs were grown under irrigation at the University of Idaho Aberdeen Research and Extension Center, Aberdeen, ID, in 1998 to 2000, and under nonirrigation at the University of Idaho Tetonia Research and Extension Center, Tetonia (Newdale), ID, in 1997 and 1999. In all locations and years, seed of parents and RILs were planted in plots using a randomized complete block design with two replicates. In addition, two plots of each parental line were randomized within each block. Plots consisted of four 2.4-m rows planted 0.36 m apart. The two middle rows of each plot were harvested with a two-row binder and threshed. Two 7.0-g samples were randomly taken from the harvested seed of each plot and were dehulled for tocopherol analysis.

Tocopherol Measurement

Dehulled whole oat grain was ground in a Retsch ZM-1 mill (Brinkman Instruments Inc., Mississauga, ON) and passed through a 0.5-mm screen. The freshly ground powder was weighed and placed in a 16 by 125 mm screw capped glass test tube. Seven milliliters of methanol was added, the contents were vortexed, and the tubes were placed on a platform shaker at low speed at room temperature for 20 min. The tubes were centrifuged at approximately 1000 × *g* for 6 to 7 min and the supernatant was decanted into a clean test tube. The pellet was extracted a second time and the supernatants pooled. The combined methanol extracts were evaporated to dryness in a speedvac (ThermoFisher Scientific, Waltham, MA) at 45°C. The residue was resuspended in 2.0 mL of hexane, vortexed vigorously then transferred to a 2-mL eppendorf tube. Immediately before high-performance liquid chromatography (HPLC) analysis the samples were centrifuged at 9000 × *g* for 10 min and an aliquot of the supernatant was analyzed by HPLC.

HPLC analysis was performed with a Spectraphysics (Newport Corp., Irvine, CA) SP8700 pump and controller interfaced with a Shimadzu (Kyotot, Japan) RF-535 fluorescence detector. The tocopherols were separated with a 5-μm, 4.6 by 250 mm silica column (Alltech, Lexington, KY) using an isocratic solvent system of 0.5% isopropanol in hexane at 1.0 mL min^{-1}. Peaks were detected with excitation at 295 nm and emission measured at 330 nm. Identification of each tocopherol was based on HPLC retention time and quantification was based on a standard curve using an α-tocopherol standard (Matrea LLC, Pleasant Gap, PA). The fluorescence intensities of the tocotrienols are essentially the same as the corresponding tocopherols (Thompson and Hatina, 1979).

Statistical Analysis

Means and variances (genotype, replicates within each location-year, and location-year) were calculated for α-tocotrienol, α-tocopherol, and total tocopherols using the JMP 6.0.2 statistical software (SAS Institute, Cary, NC). Based on significant genotype × location-year interactions ($P < 0.0001$; ANOVA) each location-year was treated as a separate trait in this study. Consequently, broad sense heritabilities were calculated for each experiment using the genotypic variance divided by the combined genotype + replicate + genotype × replicate + error

variances, as modified to included the replication variance from the method described by Zhu and Kaeppler (2003):

$$h^2 = \frac{\sigma^2_g}{\sigma^2_g + \sigma^2_r + \sigma^2_{g\times r} + \sigma^2_e} \quad [1]$$

QTL Mapping

Variability in α-tocotrienol, α-tocopherol, and total tocopherol levels among the OT RILs in each location-year were quantitatively mapped using WinQTL Cartographer (Wang et al., 2005). Quantitative trait locus analysis was done using the adjusted OT linkage map defined by Jackson et al. (2007) with the addition of mapped crown rust (caused by *Puccinia coronata* Corda) resistance phenotypic markers (Hoffman et al., 2006; Jackson et al., 2007). To detect QTLs for each location-year, composite interval mapping (CIM) (Zeng, 1994) was performed for each measurement. Cofactors for CIM were selected using forward regression with a significance threshold of $P = 0.05$. Significant QTLs were determined using experiment-wise significance levels established by running 1000 permutations for all traits, $\alpha = 0.05$ (Churchill and Doerge, 1994). Quantitative trait locus intervals were assigned based on the area within 1 LOD score of the QTL peak. Multiple interval mapping was used to estimate the total phenotypic variance explained by the QTLs detected in each location-year by using marker and linkage group positions of QTLs identified with CIM.

RESULTS AND DISCUSSION

Descriptive Statistics

Differences in mean α-tocotrienol, α-tocopherol, and total tocopherol content between Ogle1040 and TAM O-301 ranged from 0.6 to 3.1, 0.4 to 1.5, and 0.3 to 4.5 mg kg^{-1}, respectively, within all locations and years (Table 1). These differences were consistent with a previous study, where standard deviations of 4.5 mg kg^{-1} for α-tocotrienol and 1.5 mg kg^{-1} for α-tocopherol were reported around means calculated from 33 oat genotypes grown in the same Aberdeen and Tetonia locations over 3 yr (Peterson et al., 2005). Peterson and Qureshi (1993) also reported ranges of 9.1 to 18.6 mg kg^{-1} and 5.5 to 9.6 mg kg^{-1} in α-tocotrienol and tocopherol, respectively, for 12 oat genotypes grown in North Dakota, Indiana, and New York. Our results and the previous reports show adequate variation in tocopherol levels among oat genotypes to justify breeding programs to improve this trait.

The overall mean tocopherol contents were significantly higher ($P < 0.05$; HSD) at Aberdeen under irrigation (α-tocotrienol = 17.4 mg kg^{-1}, α-tocopherol = 8.8 mg kg^{-1}, and total tocopherols = 27.1 mg kg^{-1}) than under nonirrigated conditions at Tetonia (α-tocotrienol = 11.5 mg kg^{-1}, α-tocopherol = 7.4 mg kg^{-1}, and total tocopherols = 18.8 mg kg^{-1}). This result was consistent with a previous study involving 30 genotypes at the same locations by Peterson et al. (2005), where the mean α-tocotrienol (20.3 mg kg^{-1}) and α-tocopherol (8.3 mg kg^{-1}) contents were higher at the irrigated Aberdeen location than α-tocotrienol (16.7 mg kg^{-1}) and α-tocopherol (7.5 mg kg^{-1}) contents at the nonirrigated Tetonia location. Additionally, in the previous study mean α-tocotrienol contents were lower under nonirrigated than under irrigated conditions at the Tetonia location in all 3 yr, but no analyses were done to determine statistical significance. Irrigated and nonirrigated plots in Tetonia were grown in close proximity, thus variability between soil type and microclimate is likely greater between tests than across replication within a test. Furthermore, yield under irrigation was higher than under nonirrigation, indicating water availability was a factor in all 3 yr. Perhaps water availability during plant development is crucial to the production of tocopherols in oat, especially α-tocotrienol, which is the most abundant. Falk et al. (2004) found that 37% of the tocopherols in barley grain are stored in the endosperm. Endosperm development is greatly affected by moisture availability during grain fill, thus water stress may lower

Table 1. Means and variance components based on α-tocotrienol, α-tocopherol, and total tocopherols contents in grain of parents and recombinant inbred lines (RILs) grown at two locations over 3 yr.

Trait	Environment, year	Mean†			h^{2}‡
		Ogle	TAM O-301	RILs	
α-Tocotrienol					
	Aberdeen, 1998	20.8 (*19–23*)	18.2 (*17–19*)	17.5 (*7–35*)	91.9
	Aberdeen, 1999	18.5 (*13–25*)	17.8 (*14–21*)	19.6 (*9–36*)	88.6
	Aberdeen, 2000	16.6 (*15–20*)	13.5 (*10–16*)	14.5 (*7–23*)	61.1
	Mean	*18.6**	*16.5*	*17.2*	
	Tetonia, 1997	9.5 (*8–12*)	10.4 (*8–14*)	9.9 (*4–17*)	92.6
	Tetonia, 1999	12.5 (*10–17*)	13.1 (*10–17*)	13.2 (*3–31*)	6.0
	Mean	*11.0*	*11.8*	*11.6*	
α-Tocopherol					
	Aberdeen, 1998	10.2 (*9–11*)	9.2 (*7–13*)	8.6 (*2–14*)	92.8
	Aberdeen, 1999	10.3 (*7–12*)	9.5 (*8–11*)	10.2 (*4–16*)	90.9
	Aberdeen, 2000	7.9 (*7–9*)	6.4 (*5–7*)	6.8 (*4–13*)	23.1
	Mean	*9.5**	*8.4*	*8.6*	
	Tetonia, 1997	7.5 (*6–8*)	6.9 (*6–8*)	7.5 (*4–12*)	95.6
	Tetonia, 1999	7.1 (*5–9*)	7.5 (*6–8*)	7.9 (*3–16*)	2.7
	Mean	*7.3*	*7.2*	*7.7*	
Total tocopherol					
	Aberdeen, 1998	34.0 (*31–37*)	30.2 (*26–35*)	28.8 (*11–54*)	94.2
	Aberdeen, 1999	28.7 (*20–36*)	27.3 (*23–31*)	29.8 (*15–47*)	90.6
	Aberdeen, 2000	24.4 (*22–28*)	19.9 (*14–23*)	21.3 (*12–31*)	24.1
	Mean	*29.1**	*25.8*	*26.6*	
	Tetonia, 1997	17.0 (*14–20*)	17.3 (*14–22*)	17.4 (*9–27*)	96.1
	Tetonia, 1999	19.6 (*16–23*)	20.6 (*16–25*)	21.0 (*5–47*)	2.8
	Mean	*18.3*	*19.0*	*19.2*	

*Indicates significant differences between means within a row ($P = 0.05$).

†Means for each tocopherol content (mg kg^{-1}) were calculated from air-dried seed. Means are followed by ranges in italics and parenthesis.

‡h^2, broad sense heritabilities as described by Zhu and Kaeppler (2003).

tocopherol production in oat. Field experiments specifically designed to test this hypothesis are needed.

Ogle1040 had significantly higher levels of each tocopherol than TAM O-301 in Aberdeen, while no significant differences were found between the parental lines in Tetonia (Table 1). One explanation could be the effect of irrigation as previously discussed. Since differences between parental lines were not significant at the Tetonia location, QTLs detected were only considered if they were confirmed in at least one Aberdeen test. Broad sense heritabilities were high in Aberdeen in 1998 and 1999 and in Tetonia in 1997, while heritabilities were low in Aberdeen in 2000 and in Tetonia in 1999 (Table 1). The reason for reduced heritabilities was the increase in replication variances, which could possibly be attributed to variability in soil fertility across replications. In Aberdeen in 1998 and 1999, supplemental nitrogen was applied at a rate of 33.6 kg ha^{-1}, whereas none was applied in 2000. Therefore, there was no "standardization" of plots for fertility since no supplemental nutrients were applied. Since nitrogen can greatly affect plant health, this might explain the variability between replications contributing to low heritabilities in Aberdeen in 2000. At Tetonia in 1999, the effect of drought stress could have exacerbated variability in soil fertility causing low heritabilities. Water- and nutrient-controlled experiments will be conducted to investigate these phenomena.

Tocopherol contents in this study were expressed in terms of milligram per kilogram of grain. Since grain composition traits can be affected by various factors including grain size and plumpness, particularly in non-adapted materials, test weights of parents and RILs were compared with two well-adapted oat cultivars (Monida and Ajay) to the test locations used in this study. Correlations were also calculated between test weights and the tocopherol content measured. No significant differences were found between Ogle (37.1 g) or TAM O-301 (38.6 g) RILs (36.6 g), Monida (39.7 g) or Ajay (37.9 g) (HSD = 5.8 g) test weights, nor were any significant correlations calculated between the different tocopherols and test weight ($P < 0.05$). This data would suggest that QTL data presented in this study were probably due to genetic factors controlling the specific traits and not factors affecting grain size and composition.

α-Tocotrienol QTL Analysis

Quantitative trait loci significantly impacting α-tocotrienol content were detected on 11 of the OT linkage groups (LGs) over all location-years, while only five QTLs were detected in more than one location-year (Table 2). Small population sizes can inflate the proportion of the phenotypic variance explained by a particular QTL leading to QTL artifacts (Allison et al., 2002; Lande and Thompson, 1990). Because only 136 RILs were tested in the present study, the eight QTLs detected in only one location-year (Supplemental Table S1) will not be considered valid until confirmed by additional tests. Of the QTLs considered valid, one major QTL on LG OT-15 associated with the TAM O-301 loci was detected in all five location-years (Table 2, Fig. 1). The QTL peaks (mean LOD = 10.1) and intervals on OT-15 in all location-years were associated with the tightly linked sequence tagged site (STS) marker Amy2D and restriction fragment length polymorphism (RFLP) markers CDO455a and CDO516a (Fig. 1). The QTL accounted for an average of 21.6% of the phenotypic variation across all location-years (Table 2). The detection of a major QTL associated with the TAM O-301 loci accounting for a large portion of the phenotypic variance in all five location-years, particularly the Aberdeen locations, seems counterintuitive because mean α-tocotrienol levels in TAM O-301 were significantly lower than for Ogle1040 across the 3 yr at Aberdeen (Table 1). The exact reason for this is unknown due to the complex interactions between minor QTLs and environments.

Quantitative trait loci on LGs OT-8, OT-20, OT-32, and OT-33 were detected in two environments (Table 2, Fig. 1). The QTL on LG OT-8 associated with Ogle1040 loci had peaks associated with the RFLP probes BCD1421a and BCD1802, and in Tetonia, 1997 and Aberdeen, 2000, respectively (Fig. 1). Although no QTLs were detected in the other three location-years, peaks just below the significance threshold limit (2.5) were apparent in both locations in 1999 (Fig. 1). The QTL on LG OT-20 associated with TAM O-301 loci had peaks associated with RFLP probes CDO1508a and RZ404a in Tetonia, 1997 and Aberdeen, 2000, respectively (Fig. 1), while the QTL detected on OT-33 associated with Ogle1040 loci had peaks associated with RFLP probes PSR129b and RZ516c in Aberdeen, 2000 and Tetonia, 1999, respectively (Fig. 1). Two independent QTLs were detected on LG OT-32 in Aberdeen, 2000 and Tetonia, 1999. The first QTL peak was associated with the amplified fragment length polymorphism (AFLP) marker e35m61-122.t, while the second peak was associated with RFLP probe BCD1280b (Fig. 1). The detection of two QTLs on the same LG affecting tocopherol content in a plant species is consistent with a previous mapping study in maize (*Zea mays* L.) where multiple QTLs affecting tocopherol production were mapped to a 50-cM region on chromosome 5 (Wong et al., 2003).

Overall, three genetic regions associated with TAM O-301 loci and two genetic regions associated with Ogle1040 loci significantly increased α-tocotrienol content in this study. In the 1997 Tetonia trial, the three QTLs on LGs OT-8, 15, and 20, and the two QTLs on OT-32 accounted for 56.3% of the phenotypic variation at the location (Table 2). In Aberdeen in 2000, the same QTLs along with one QTL on OT-33 account for 56.3% of the phenotypic variation, while only the QTLs on OT-15

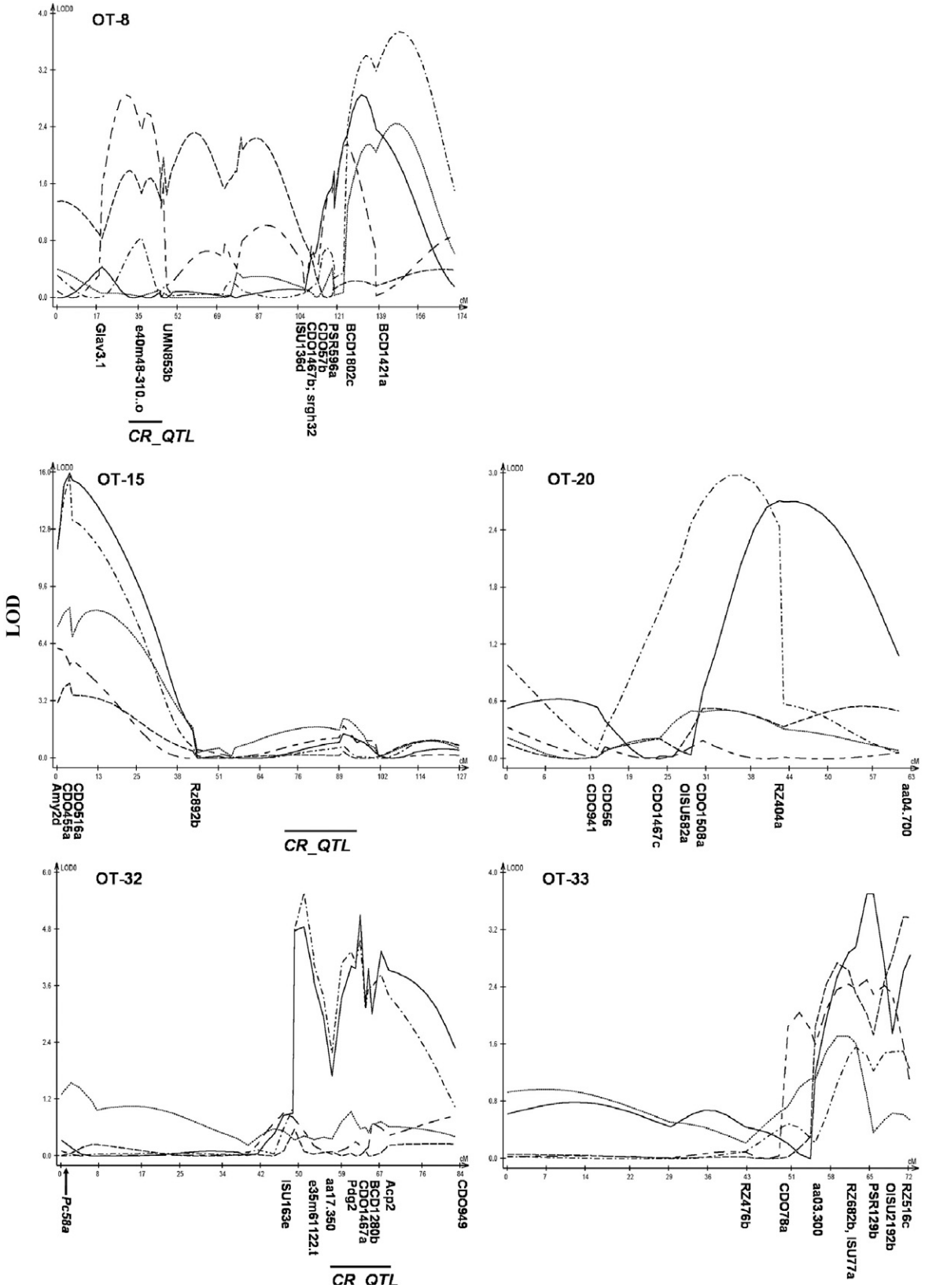

Figure 1. Quantitative trait loci (QTLs) controlling α-tocotrienol contents in Ogle and TAM O-301 validated in at least two environments. QTLs were detected using WinQTL Cartographer CIM with a significant LOD (*y* axis) threshold of 2.5 based on 1000 permutations and a type I error of 5%. Traits were based on α-tocotrienol contents from parents and 136 Ogle/TAM O-301 RIL seed harvested in Tetonia, ID 1997 (-··-), Aberdeen, ID 1998 (–), Aberdeen, ID 1999 (··), Tetonia, ID 1999 (--), and Aberdeen, ID 2000 (solid line). Genetic markers (e-axis) including restricted fragment length polymorphism (RFLP) probes (BCD, CDO, ISU, OISU, PSR, RZ, srgh, and UMN) and amplified fragment length polymorphism (AFLP) (e and aa), isozyme (Acp and Pgd), and intron (Glav and Amy) markers were mapped using Mapmaker 3.0/EXP with a LOD of 3.0. In addition, phenotypic markers for crown rust resistance genes *Pc* and QTL intervals (*CR_QTL*–) were also placed on the charts.

and 33 were detected in 1998 accounting for 18.9% of the phenotypic variation (Table 2).

α-Tocopherol QTL Analysis

Quantitative trait loci significantly affecting α-tocopherol content were detected on 10 of the OT LGs, five of which were detected in more than one location-year (Table 3). Since QTL analysis using small population sizes can produce QTL artifacts, the five QTLs not detected in more than one location-year (Supplemental Table) were not considered valid in this study. Of the valid QTLs, one on LG OT 34 associated with Ogle1040 loci was detected in all five location-years (Table 3, Fig. 2). The QTL had peaks in all five location-years (mean LOD 3.6) associated with the RFLP probe RZ395b and intervals between RFLP probes RZ69a and RZ404c (Fig. 2). This QTL accounted for an average of 9.7% of the phenotypic variation across all five location-years, with the highest portion of the variation being accounted for in the Tetonia, 1999 environment (Table 3).

Quantitative trait loci associated with TAM O-301 loci were detected on LG OT 30 in four location-years (Table 3, Fig. 2). Peaks of the QTLs were associated with the three different RFLP probes including CDO516b in 1999 at Tetonia; between CDO516b and CDO772 in 1997 at Tetonia; and 1998 at Aberdeen; and with BCD1840a in Aberdeen, 1999 (Fig. 2). Although the peak in the 1999 Aberdeen location-year was associated with an RFLP probe approximately 16 to 26 cM from the peaks of other location-years, a second peak (LOD 2.6), accounting for less of the phenotypic variance was detected close to the same RFLP probe (CDO516b) associated with the peak in 1999 at Tetonia (Fig. 2).

Based on the latter data, all four QTL peaks would be associated with RFLP probes between CDO516b and CDO772 (Fig. 2). It is likely that only one QTL exists on LG OT-30, since the reason for the differences in QTL peak associations between location-years can be explained by the lack of genotypic markers in this region and/or possible errors in phenotypic and genotypic data.

A second group of QTLs associated with Ogle1040 loci were detected on LG OT-16 in four location-years (Table 3, Fig. 2). Of these, two QTLs were detected in the 1999 Aberdeen location-year, one with a peak associated with the resistance gene analog marker srgh8a (LOD 4.3) and the other with a peak associated with the RFLP probe CDO1090a (LOD 4.0). The QTL peak associate with CDO1090a was also detected in the 2000 Aberdeen location (Fig. 2), while a third QTL peak associated with the AFLP marker e35m61-210.o was detected in the 1997 Tetonia and 1998 Aberdeen location-years (Fig. 2). Unlike the QTL on LG OT-30, it is probable that two to three independent QTLs affecting α-tocopherol content exists on OT-16 because (i) there was good genotypic coverage on the LG and (ii) QTL peaks associated with e35m61-210.o (LOD 2.0) were present just below the significance threshold in the 1999 and 2000 Aberdeen location-years (Fig. 2). The presence of multiple QTLs affecting α-tocopherol content on this LG is consistent with the multiple QTLs affecting α-tocotrienol content on OT-32 as well as the previous report of multiple QTLs on chromosome 5 in maize affecting various tocopherol components (Wong et al., 2003).

Additional QTLs associated with Ogle1040 loci were detected on LG

Table 2. Quantitative trait locus (QTL) analysis summary for α-tocotrienol grain content (mg kg^{-1}) in Ogle × TAM O-301 $F_{6:8}$ recombinant inbred lines grown in two locations over 3 yr.

Experiment (environment, year)	Linkage group	QTL marker† (peak/interval)	LOD‡	R^2§	Additive¶
				%	
Tetonia, 1997					
	OT-8	PSR596a (147.5/127–162)	3.7	11.4	0.8
	OT-15	Amy2d (3.7/2–4)	15.8	33.5	–1.3
	OT-20	CDO1467c (36.4/26–43)	3.0	6.5	–0.6
	OT-32	e35m61-122.t (62.9/60–64)	4.6	8.0	–0.6
	OT-32	Adh2c (51.0/49–53)	5.6	11.4	–0.8
				Total (%)¶ 56.3	
Aberdeen, 1998					
	OT-15	Amy2d (3.8/0–16)	4.2	9.2	–1.0
	OT-33	PSR129b (71.0/67–72)	3.4	7.8	1.0
				Total (%) 18.9	
Aberdeen, 1999					
	OT-15	Amy2d (3.8/0–4)	8.4	19.0	–1.6
Tetonia, 1999					
	OT-15	Amy2d (0.0/0.0–4)	6.2	13.5	–0.9
Aberdeen, 2000					
	OT-8	PSR596a (131.2/122–147)	2.9	5.5	0.7
	OT-15	Amy2d (1.9/2–10)	16.0	32.6	–1.7
	OT-20	CDO1508a (42.4/35–57)	2.7	4.8	–0.6
	OT-32	Adh2c (51.0/49–53)	4.9	9.2	–0.9
	OT-32	A17.350 (62.9/62–64)	5.1	8.7	–0.9
	OT-33	A03.300 (64.5/60–67	3.7	6.5	0.73
				Total (%) 56.3	

†Name of the flanking marker to the left of the QTL peak and interval (cM)

‡QTLs were detected using WinQTL Cartographer CIM and were based on a LOD threshold of 2.5 (1000 permutations and a type I error of 5%).

§Percentage of the phenotypic variation explained by the QTL.

¶Total phenotypic variation explained by all QTLs was calculated using WinQTL Cartographer MIM.

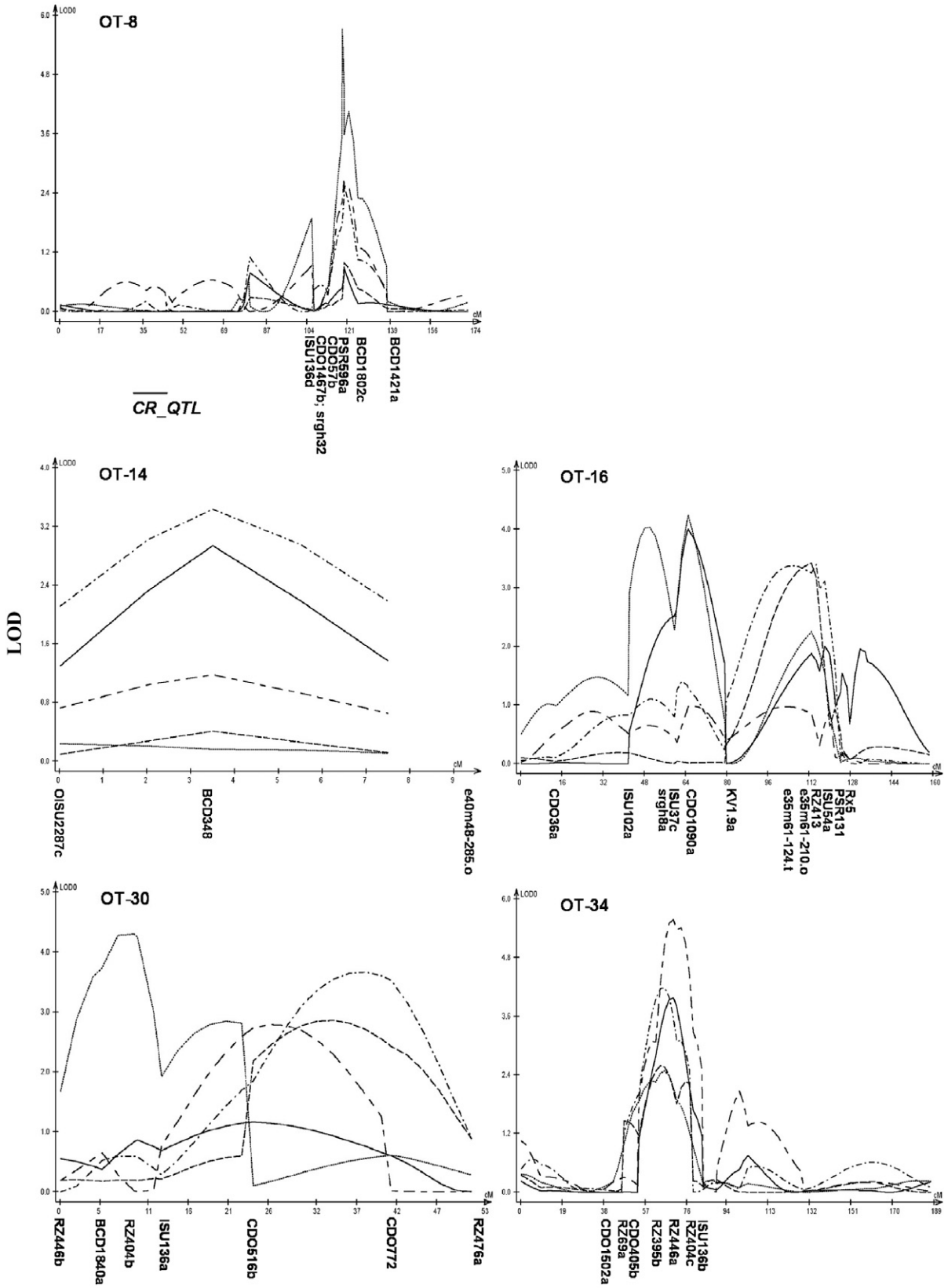

Figure 2. Quantitative trait loci (QTLs) controlling α-tocopherol contents in Ogle and TAM O-301 validated in at least two environments. QTLs were detected using WinQTL Cartographer CIM with a significant LOD (*y* axis) threshold of 2.5 based on 1000 permutations and a type I error of 5%. Traits were based on α-tocopherol contents from parents and 136 Ogle/TAM O-301 RIL seed harvested in Tetonia, ID 1997 (-·-), Aberdeen, ID 1998 (–), Aberdeen, ID 1999 (··), Tetonia, ID 1999 (--), and Aberdeen, ID 2000 (solid line). Genetic markers (e-axis) including restricted fragment length polymorphism (RFLP) probes (BCD, CDO, ISU, OISU, PSR, RZ, and srgh) and amplified fragment length polymorphism (AFLP) (e and aa), isozyme (Acp and Pgd), and sequence tagged site (STS) (KV and Px) markers were mapped using Mapmaker 3.0/EXP with a LOD of 3.0. In addition, phenotypic markers for crown rust resistance QTL intervals (*CR_QTL–*) were also placed on the charts.

Table 3. Quantitative trait locus (QTL) analysis summary for α-tocopherol grain content (mg kg^{-1}) in Ogle × TAM O-301 $F_{6:8}$ recombinant inbred (RI) lines grown in two locations over 3 yr.

Experiment (environment, year)	Linkage group	QTL marker† (peak/interval)	LOD‡	R^2§	Additive¶
				%	
Tetonia, 1997					
	OT-8	CDO1467b (119.4/117–123)	2.6	5.3	0.3
	OT-16	kv1.9a (114.6/89–120)	3.4	7.6	0.4
	OT-30	CDO516b (38.0/28–45)	3.7	8.2	–0.4
	OT-34	RZ69a (65.6/59–71)	4.2	10.4	0.5
	OT-14	OISU2287c (3.5/0–7)	3.4	7.3	0.4
				Total (%)¶ 46.6	
Aberdeen, 1998					
	OT-16	kv1.9a (112.6/97–117)	3.4	8.2	0.5
	OT-30	CDO241b (34.0/24–47)	2.9	8.9	–0.5
	OT-34	RZ69a (63.6/51–78)	2.6	7.0	0.5
				Total (%) 25.3	
Aberdeen, 1999					
	OT-8	CDO1467b (118.8/118–119)	5.7	13.6	0.8
	OT-16	ISU102a (50.0/42–56)	4.0	15.0	0.8
	OT-16	srgh8a (64.9/61–72)	4.3	9.8	0.6
	OT-30	RZ446b (9.2/3.2–11)	4.3	10.0	–0.6
	OT-34	RZ69a (65.6/50–78)	2.5	6.1	0.5
				Total (%) 37.0	
Tetonia, 1999					
	OT-8	CDO1467b (119.4/116–124)	2.7	6.4	0.4
	OT-30	ISU136a (26.0/18–37)	2.8	7.5	–0.4
	OT-34	RZ395a (69.6/65–77)	5.6	15.4	0.7
				Total (%) 28.3	
Aberdeen, 2000					
	OT-16	ISU102a(64.9/61–73)	4.0	8.8	0.4
	OT-34	CDO405b (69.6/65–73)	4.0	9.7	0.4
	OT-14	OISU2287c (3.5/1–6)	3.0	6.4	0.3
				Total (%) 28.9	

†Name of the flanking marker to the left of the QTL peak and interval (cM)

‡QTLs were detected using WinQTL Cartographer CIM and were based on a LOD threshold of 2.5 (1000 permutations and a type I error of 5%).

§Percentage of the phenotypic variation explained by the QTL.

¶Total phenotypic variation explained by all QTLs was calculated using WinQTL Cartographer MIM.

OT-8 in three location-years and on LG OT-14 in two location-years (Table 3, Fig. 2). The QTL peaks on OT-8 were associated with RFLP probes PSR596a and BCD1802c, while the peaks on OT-14 were associated with RFLP probe BCD348 (Fig. 2).

Unlike the detection of a major QTL accounting for a large portion of the α-tocotrienol variation, at least three QTLs accounted for a majority of the overall α-tocopherol variation. In Tetonia 1999, QTLs on LGs OT-8, OT-30, and OT-34 accounted for 28.3% of the phenotypic variation, while the same QTLs along with QTLs on OT-14 and OT-16 accounted for 46.6% of the variation in the 1997 Tetonia trial (Table 3). In the 1998 Aberdeen trial, QTLs on LGs OT-16, OT-30, and OT-34 accounted for 25.3% of the phenotypic variation, while the same QTLs along with additional QTLs on OT-16 and OT-8 accounted for 37.0% of the variation at Aberdeen in 1999. In 2000 at Aberdeen, QTLs on LGs OT14, OT-16, and OT-34 accounted for 28.9% of the phenotypic variation.

Total tocopherol QTL Analysis

Quantitative trait loci affecting total tocopherol content were detected on nine of the OT LGs, however, eight were only detected in one location-year (Supplemental Table) and were not considered valid in this study. Of the valid QTLs, one on LG OT-15 associated with TAM O-301 loci and one on LG OT-8 and on LG OT-33 associated with Ogle1040 loci were detected in multiple location-years (Table 4, Fig. 3). All three QTLs were detected in the same location-years and had roughly the same peaks and intervals as the QTLs for α-tocotrienol content (Fig. 3). Since α-tocotrienol is the most abundant tocopherol found in oat (Barnes, 1983) and together α-tocotrienol and α-tocopherol accounted for 99.2% of the total tocopherols measured (Table 1), it is probable that the total tocopherol QTLs are the same as the α-tocotrienol QTLs. The only other valid QTL was associated with Ogle loci and detected on LG OT-31 (Table 4, Fig. 3). The QTL was associated with the AFLP marker aa.04.810 and the RFLP probe BCD1643a in the 1999 and 2000 Aberdeen trials, respectively (Fig. 3). Since all eight tocopherol isomers have been identified in oat (Lásztity et al., 1980) but only two specific isomers were measured in this study, it is possible that either one or two QTLs on LG OT-31 associated with Ogle1040 loci affect one of the remaining six isomers.

Potential Impact and Application to Breeding Programs

The detection of QTLs contributing to increased α-tocotrienol and α-tocopherol content in both parents was expected due to the presence of transgressive segregates in all five location-years for both traits (Table 1). Across Aberdeen locations, some OT RILs had higher mean α-tocotrienol and α-tocopherol than either parental line including the highest mean α-tocotrienol (20.8 mg kg^{-1}) or α-tocopherol (10.3 mg kg^{-1}) from Ogle1040 in Aberdeen, 1998 and 1999, respectively. Examples of transgressive segregation included the OT RILs OT-109

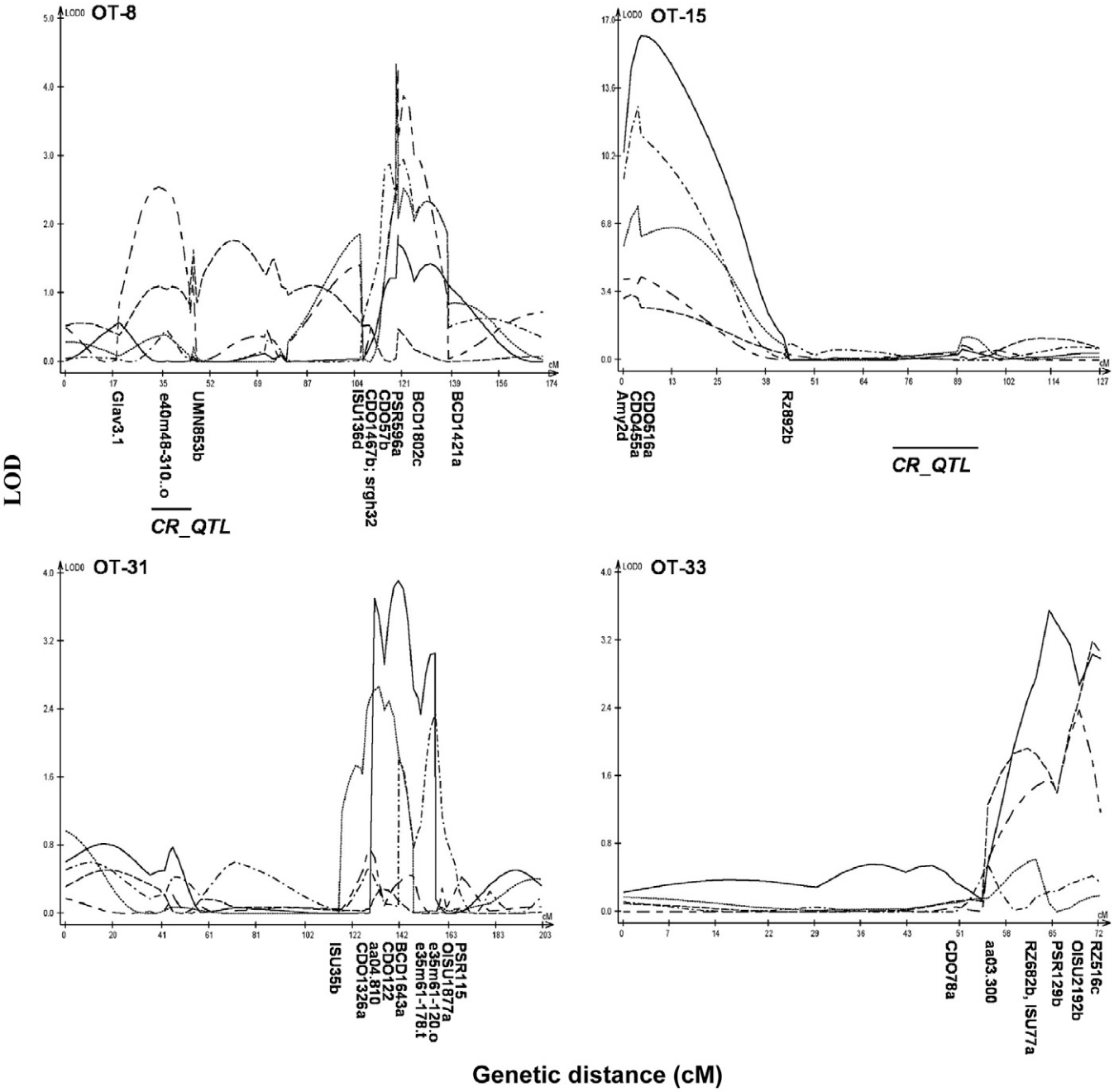

Figure 3. Quantitative trait loci (QTLs) controlling total tocopherol contents in Ogle and TAM O-301 validated in at least two environments. QTLs were detected using WinQTL Cartographer CIM with a significant LOD (*y* axis) threshold of 2.5 based on 1000 permutations and a type I error of 5%. Traits were based on total tocopherol contents from parents and 136 Ogle/TAM O-301 RIL seed harvested in Tetonia, ID 1997 (-··-), Aberdeen, ID 1998 (–), Aberdeen, ID 1999 (··), Tetonia, ID 1999 (--), and Aberdeen, ID 2000 (solid line). Genetic markers (e-axis) including restricted fragment length polymorphism (RFLP) probes (BCD, CDO, ISU, OISU, PSR, RZ, srgh, and UMN) and amplified fragment length polymorphism (AFLP) (e and aa), isozyme (Acp and Pgd), and intron (Glav and Amy) markers were mapped using Mapmaker 3.0/EXP with a LOD of 3.0. In addition, phenotypic markers for crown rust resistance genes *Pc* and QTL intervals (*CR_QTL–*) were also placed on the charts.

and OT-126, which had mean α-tocotrienol contents 23% (23.72 and 24.32 mg kg^{-1}) greater than Ogle1040, while the RILs OT-73 and OT-37 had mean α-tocopherol contents 33% (13.72 mg kg^{-1}) and 23% (12.25 mg kg^{-1}) greater than Ogle1040. Of the transgressive segregant RILs, OT-126 had the highest contents of both α-tocotrienol (24.32 mg kg^{-1}) and α-tocopherol (12.43 mg kg^{-1}) across all locations. Our detection of transgressive segregants was consistent with findings in an oilseed rape (*Brassica napus* L.) population where the maximum level of α-tocopherol in the doubled haploid lines was 15.2% greater than either parental line (Marwede et al., 2005).

The highest single location-year mean content of α-tocotrienol measured in this study was 28.42 mg kg^{-1},

while the highest mean content of α-tocopherol measured was 15.87 mg kg^{-1}. These values were 4 and 6.8 mg kg^{-1} greater than the highest mean α-tocotrienol and α-tocopherol measurements, respectively, from previous reports (Peterson and Qureshi, 1993; Peterson et al., 2005). Based on this data and the high heritabilities calculated in this study (Table 1), it appears considerable gain can be made in improving α-tocotrienol and α-tocopherol levels in oat.

Major deterrents to increasing tocopherol levels in oat are the time, labor, and expense required for measurement (Rocheford et al., 2002). These issues make routine screening of numerous breeding lines impractical. In the current study, we have identified one QTL on OT-15 accounting for a significant portion of the overall α-tocotrienol variation in all five location-years tested. The QTL peak and interval were tightly linked to the STS marker for the alpha amylase gene Amy2d (Willmott et al., 1998) and the RFLP probes CDO 455a and CDO516a (Fig. 1). Since the STS marker is closely linked to the QTL peak, it should be useful for marker-assisted selection (MAS). To further improve the MAS potential for this QTL, existing sequence information for both RFLP probes can be used to develop STS markers and/or single nucleotide polymorphism (SNP) markers. The development of SNP markers from RFLP sequence information has been successful for oat crown rust resistance genes (Chen et al., 2006), thus work should be done to develop additional markers for this important region. In addition to this QTL, five QTLs affecting α-tocotrienol content and six QTLs affecting α-tocopherol content were identified. The most interesting of these were the QTL detected on OT-34 affecting α-tocopherol in all five location-years and the QTL detected on OT-8 affecting both tocopherols. Since these QTLs clearly affected respective tocopherol contents, and their peaks and intervals were associated with several tightly linked RFLP probes, polymerase chain reaction (PCR)-based STS or SNP markers should be developed to facilitate MAS. Furthermore, two QTL regions on OT-32 and on OT-16 clearly controlled α-tocotrienol and α-tocopherol, respectively. The QTL regions on OT-32 were between the RFLP probe ISU163a and the AFLP marker e35m61-122.t, and the AFLP marker aa17.350 and the isozyme marker Pdg2. The latter region is also associated with a QTL reducing crown rust resistance (Jackson et al., 2007). The QTLs on OT-16 were between the sorghum (*Sorghum* spp.) resistance gene analog marker srgh8a and the STS marker for Beta-1-Hordein sequence KV1.9a (Tragoonrung et al., 1992); and the KV1.9a marker and the AFLP marker e35m61-210, respectively. Since the conversion of AFLP markers into PCR-based markers has been successful (Chen et al., 2007) and only five of the eight markers linked to the aforementioned QTL on OT-16 are not PCR-based, it appears that practical MAS for these regions is feasible. Although QTL peaks and intervals of the remaining peaks were also tightly linked to RFLP probes, multiple peaks or variation in QTL location made overall target regions unclear. Thus before investing considerable resources in conversion of tightly linked RFLP probe sequence into PCR-based markers for MAS, additional work should be done to clarify exact locations.

To our knowledge, this is the first study identifying QTLs affecting variation of tocopherols in oat. This study has identified QTLs accounting for significant portions of the variation for the two major tocopherols in oat. The next step would be to develop PCR-based markers from RFLP probes and AFLP markers linked to the QTL identified in this study. These developed markers

Table 4. Quantitative trait locus (QTL) analysis summary for the total tocopherol grain content (mg kg^{-1}) in Ogle × TAM O-301 $F_{6:8}$ recombinant inbred lines grown in two locations over 3 yr.

Experiment (environment, year)	Linkage group	QTL marker† (peak/interval)	LOD‡	R^2§	Additive¶
				%	
Tetonia, 1997					
	OT-8	CDO1467b (121.4/113–137)	3.0	6.1	0.8
	OT-15	Amy2d (3.8/3–5)	12.7	27.0	–1.7
				Total (%)¶ 31.4	
Aberdeen, 1998					
	OT-15	Amy2d (2.0/0–17)	3.3	7.8	–1.4
	OT-33	OISU192b (71.0/70–72)	3.2	8.0	3.2
				Total (%) 13.6	
Aberdeen, 1999					
	OT-8	srgh32 (118.7/118–119)	4.3	9.1	1.7
	OT-15	Amy2d (3.8/2–5)	7.7	17.0	–2.0
	OT-31	ISU35b (133.2/126–144)	2.7	5.7	–1.2
				Total (%) 32.8	
Tetonia, 1999					
	OT-8	srgh32 (119.4/119–120)	4.3	10.0	1.4
	OT-15	Amy2d (4.6/0–13)	4.2	9.3	–1.1
				Total (%) 21.4	
Aberdeen, 2000					
	OT-15	Amy2d (4.6/3–11)	16.2	32.5	–2.1
	OT-31	CDO1326a (64.5/131–147)	3.6	6.2	0.9
	OT-33	aa03.300 (64.5/62–72)	3.6	6.2	0.9
				Total (%) 38.6	

†Name of the flanking marker to the left of the QTL peak and interval (cM).

‡QTLs were detected using WinQTL Cartographer CIM and were based on a LOD threshold of 2.5 (1000 permutations and a type I error of 5%).

§Percentage of the phenotypic variation explained by the QTL.

¶Total phenotypic variation explained by all QTLs was calculated using WinQTL Cartographer MIM.

can then be utilized with the existing PCR-based markers to validate each QTL position in this and other genetic backgrounds to determine the QTL usefulness. Once this work is complete, the results should provide the needed resources to improve α-tocotrienol and α-tocopherol content in oat breeding programs using MAS. In addition, the exact molecular or physiological mechanisms controlling tocopherol content in oat requires more investigation. The structural genes on the synthetic pathway of tocopherols are relatively clear (Hofius and Sonnewald, 2003). However, the contribution of each gene to tocopherol content, the epistatic effects, and the overall environmental sensitivity of each gene are still unknown. Our genetic study of the two major tocopherols including evaluating the genotypes and mapping the QTLs are important steps in elucidating the molecular mechanisms.

Acknowledgments

The authors thank Irene Shackelford and Lauri Herrin for their technical expertise in the field and laboratory experiments and with data collection. We are also grateful to Jim Whitmore, Denise Wedel, Randy Gamble, and the staff of the University of Idaho Research and Extension Center for field plot maintenance. This research was supported in part by the Quaker Oats Company, a unit of PepsiCo Beverages & Foods. The authors dedicate this manuscript to the late Dr. Dave L. Hoffman whose efforts were the foundation of this research.

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